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A single nucleotide polymorphism in the matrix metalloproteinase-1 and interleukin-8 gene promoter predicts poor prognosis in tongue cancer

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Abstract

Objective: Matrix metalloproteinase-1 (MMP-1) and interleukin-8 (IL-8) play an important role in cancer development and metastasis. There is a single nucleotide polymorphism (SNP) located in the promoter region of *MMP-1* and *IL-8* that regulates gene expression. *MMP-1* −1607 *2G/2G* and *IL-8* −*251 A/A* genotypes enhance transcriptional activity and may be associated with increased risk in malignant tumors. We therefore evaluated the impact of these SNPs in tongue squamous cell carcinoma (SCC).
Methods: In this study, we genotyped 69 tongue SCC patients. The expression of MMP-1 and IL-8 in tongue SCC patients was analyzed by immunohistochemistry.
Results: We found a significant difference in *IL-8 A/A* genotypes with nodal recurrence ($P = 0.0068$). An analysis of disease-free survival rates showed that the presence of both *MMP-1 2G/2G* and *IL-8 A/A* genotypes was associated with a particularly poor prognosis ($P = 0.0032$) and was an independent prognostic factor ($P = 0.001$). The expression of MMP-1 was significantly correlated with the frequency of *MMP-1 2G/2G* genotypes ($P = 0.049$).
Conclusion: These results suggest that SNP in the promoter region of *MMP-1* and *IL-8* plays an important role in tumor progression and recurrence through its expression in tongue SCC.

Keywords: Single nucleotide polymorphism; Matrix metalloproteinase-1; Interleukin-8; Oral cancer; Immunohistochemistry

1. Introduction

Malignant tumors have the ability to invade normal tissue and spread to distant sites, giving rise to metastasis, the major factors in the morbidity and mortality of cancer. The extracellular matrix acts as a structural support network within tissues and as a barrier to cell migration. Matrix degradation is mediated by the concerted action of several proteinases, including matrix metalloproteinase (MMPs), a family of enzymes [1]. Several recent studies have suggested new functional roles for MMPs in supporting tumor growth, modulating the extracellular matrix, regulating the availability of growth factors, and facilitating angiogenesis [2–4]. Matrix metalloproteinase-1 (MMP-1) has been implicated in tumor invasion and metastasis [5] as its specific ability to degrade type-I collagen, which is the most abundant substrate in the tumor surrounding stroma. Many reports have shown a significant negative correlation between the expression of MMP-1 and survival in advanced cancers [6–8].

The presence of a *MMP-1 1G/2G* polymorphism in the promoter region of the *MMP-1* gene at −1607 bp (GenBank AF023338 2767) affects the transcriptional level of *MMP-1* in both normal fibroblasts and in melanoma cells [9]. The *MMP-1 2G* polymorphism is positively correlated with an increased risk for developing lung cancer, ovarian cancer,

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colorectal cancer, and head and neck cancer [10–13]. These data establish a potential role for the *2G* polymorphism in cancer development.

Angiogenesis, or neovascularization, is the formation of new blood vessels that occurs during embryogenesis, wound healing, and tumor progression. In addition to the fact that angiogenesis is an indispensable phenomenon for the growth of tumors, the close association of angiogenesis with tumor metastasis has been well documented [14,15]. In recent studies, interleukin-8 (IL-8) has been shown to be a potent angiogenic factor implicated in tumor growth, metastasis and poor prognosis [16–19].

Promoter regions of a number of cytokine genes contain polymorphisms that directly influence cytokine production [20]. In the *IL-8* −251 (GenBank AF385628 3470) *A/T* polymorphism, the *A* allele tended to be associated with increased IL-8 production [21]. The frequency of *IL-8 T/T* genotypes was less than that of the *IL-8 A/T* or *A/A* genotype in prostate cancer patients [22].

We have been working on the regulation of invasion and metastasis in head and neck cancers, and revealed the important role of both MMPs and angiogenic factors. It is generally accepted that overexpression of such factors predicts a poor outcome in patients with various malignant tumors; however, studies on the expression level are always accompanied by the problem of reproducibility of the determined data. Analysis of genetic events, such as genotyping, provides quite stable data; however, the results may not be as impressive as the data from expression profiles. From this viewpoint, we evaluated the risk and prognostic value of *MMP-1* −1607 *1G/2G* and *IL-8* −251 *A/T* polymorphisms in tongue squamous cell carcinoma (SCC). Moreover, we examined the expression of MMP-1 and IL-8 proteins by immunohistochemical examination, and the relationship between immunohistochemical examination and genotype data was also evaluated.

2. Materials and methods

2.1. Study subjects

This study consisted of 69 patients with tongue SCC and 91 healthy controls, and all subjects were ethnic Japanese. The healthy controls consisted of staff, medical students, and inpatients of Kanazawa University Hospital without a previous diagnosis of malignant neoplasm. The data of tongue SCC patients in this study were obtained from medical records. From April 1983 to January 2000, 99 tongue SCC patients without metastasis were diagnosed and treated at the Department of Otolaryngology, Kanazawa University Hospital and Toyama City Hospital in Japan. Among them, 69 patients from whom DNA samples were available were recruited in this study. Clinical status was determined with the 1997 UICC/AJCC staging system [23]. Sixty-five patients were treated with surgery. Therapeutic neck dissection was performed on 17 patients who had clinically positive nodes. Selective neck dissection was performed on 23 patients with clinically negative nodes who had T2 or more advanced tumor status. Neck dissection was not performed on 23 patients with stage I disease. Postoperative radiotherapy was administered to the neck of patients with pathologically positive lymph node metastasis. Although the tumor-free surgical margin was confirmed from frozen sections of the primary tumor, four patients were shown to have positive margins by using permanent pathological specimens. These patients received postoperative irradiation. Four patients underwent chemotherapy with or without radiation. The median follow-up time was 46 months (range, 4–96 months). Disease-free survival time was calculated from the date of treatment until the time of recurrence, defined as disease recurrence at the same site or the detection of metastases, including recurrence in the neck lymph nodes.

2.2. Sample preparation

Surgical specimens were fixed with 10% formalin, embedded in paraffin, and the primary tumors were examined by immunohistochemical analysis. Paraffin-embedded specimens were retrieved from the surgical pathology files of the Pathology Section of Kanazawa University Hospital and Toyama City Hospital in Japan.

DNA samples of tongue SCC were extracted from paraffin blocks and those of healthy individuals from whole blood samples. This study design was approved by the Ethics Committee of Kanazawa University.

2.3. DNA extraction

DNA from whole blood or paraffin-embedded tissue was extracted with a MagExtractor System MFX-2000 (TOYOBO, Osaka, Japan); DNA from paraffin-embedded tissue was also extracted with a modified protocol [24]. In brief, 10 mm × 10 mm paraffin sections were incubated with 1 ml xylene at 55 °C for 15 min. After centrifugation, the supernatant fluid was discarded. The pellet was washed twice with 1 ml of 1/1 xylene/100% ethanol and twice with 100% ethanol. Pellets were incubated with 180 ml buffer ATL and 20 ml proteinase K at 55 °C for 48 h with intermittent vortexing, after which the manufacturer's protocol was followed.

2.4. Genetic analysis

Genotyping was accomplished using PCR followed by melting curve analysis with specific fluorescent hybridization probes in a Light Cycler System (Roshe, Mannheim, Germany) [25,26].

To detect *MMP-1* −1607 (GenBank AF023338 2767) *1G/2G* genotypes, a 230-bp fragment was amplified by primer extension reaction (PCR) using forward and reverse primers,